

Supplementary Table S1 and Figures S1-S8

CDK4/6 and IGF1 receptor inhibitors synergize to suppress the growth of p16INK4A-deficient pancreatic cancers

Andreas M. Heilmann, Rushika M. Perera, Veronika Ecker, Brandon N. Nicolay, Nabeel Bardeesy, Cyril H. Benes, and Nicholas J. Dyson

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Table S1. Natural log IC50s (μM) for IGF1R/IR inhibitors and PD-0332991 in PDAC cell lines.

Cell Line	<i>CDKN2A</i>	BMS-754807	OSI-906	GSK1904529A	PD-0332991
PANC-10-05	wt::0<cn<8	-1.503601	1.529179	2.555885	3.3242
PANC-08-13	p.0?::0	-4.64351	-0.238705	3.519599	3.0564
BxPC-3	p.0?::0	0.340139	3.943117	3.55466	1.8363
Capan-2	wt::0<cn<8	-1.563187	2.357756	3.610405	3.0465
HuP-T3	p.0?::0	0.55552	3.213053	3.768508	1.6462
MIA-PaCa-2	p.0?::0	-1.347752	2.012654	3.978953	0.86051
PANC-03-27	p.0?::0	0.155327	2.442733	3.984894	4.0374
PSN1	p.0?::0	-2.295558	1.662574	3.986342	3.9403
AsPC-1	p.L78fs*41::0<cn<8	2.93109	3.663701	4.01949	-1.3762
KP-4	p.0?::0	1.34036	4.842081	4.044681	
HPAF-II	p.R29fs*9::0<cn<8	2.579806	2.945641	4.29352	3.5116
CFPAC-1	wt::0<cn<8	0.707539	3.708023	4.307708	1.7991
CAPAN-1	p.0?::0	0.304247	1.69856	4.35411	2.0275
SW1990	p.0?::0	2.987129	3.913512	4.532534	2.0852
YAPC	p.0?::0	3.1204	1.715303	4.732241	3.147
HuP-T4	p.0?::0	0.5569	5.312025	4.776506	2.8915
MZ1-PC	p.R80*::0<cn<8	-1.531715	2.362118	4.793137	1.4316

Supplementary Figure S1

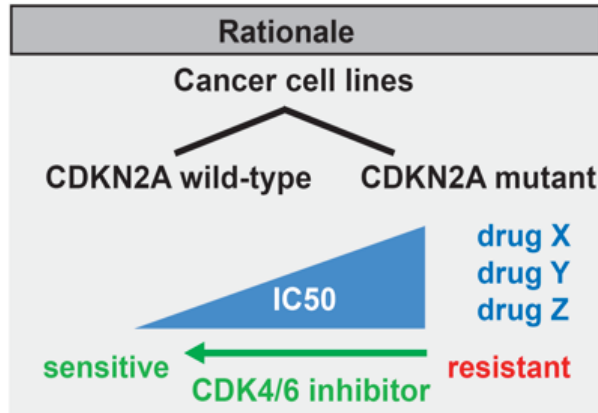


Figure S1. Rationale for combining the CDK4/6 inhibitor and drugs with decreased sensitivity in *CDKN2A*-mutant cell lines. The drugs (X, Y, and Z) were selected based on a tissue type specific comparison of sensitivities by *CDKN2A* mutation status. To this goal, sensitivities across all cancer cell lines determined by the Center for Molecular Therapeutics (MGH Cancer Center, www.cancerrxgene.org) were first stratified by their tissue of origin and subsequently compared between *CDKN2A*-mutant and wild-type cell lines. Drugs with decreased sensitivity in *CDKN2A*-mutant lines were experimentally tested for synergy with the CDK4/6 inhibitor PD-0332991 in this setting.

Supplementary Figure S2

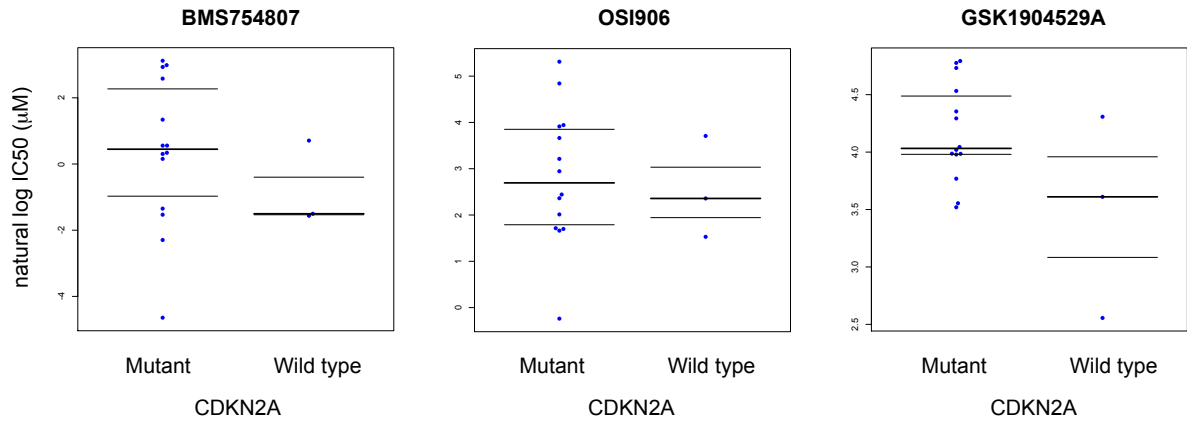


Figure S2. Distribution of IGF1R/IR inhibitor sensitivities in CDKN2A wild-type and mutant PDAC cell lines.

Strip charts show natural log IC50s (μM) with quartiles. IC50 values are listed in Table S1.

Supplementary Figure S3

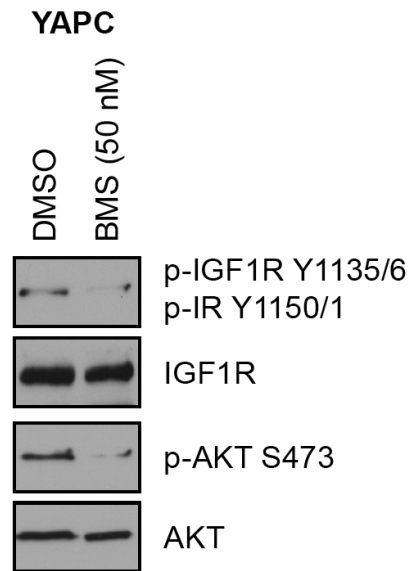


Figure S3. Target inhibition by the IGF1R/IR inhibitor BMS-754807.

YAPC cells were starved over night in serum-free medium and treated for 1 h with 50 nM BMS-754807 (BMS) or DMSO in 10% serum. Cell lysates were analyzed by Western blotting with the indicated antibodies (N=2).

Supplementary Figure S4

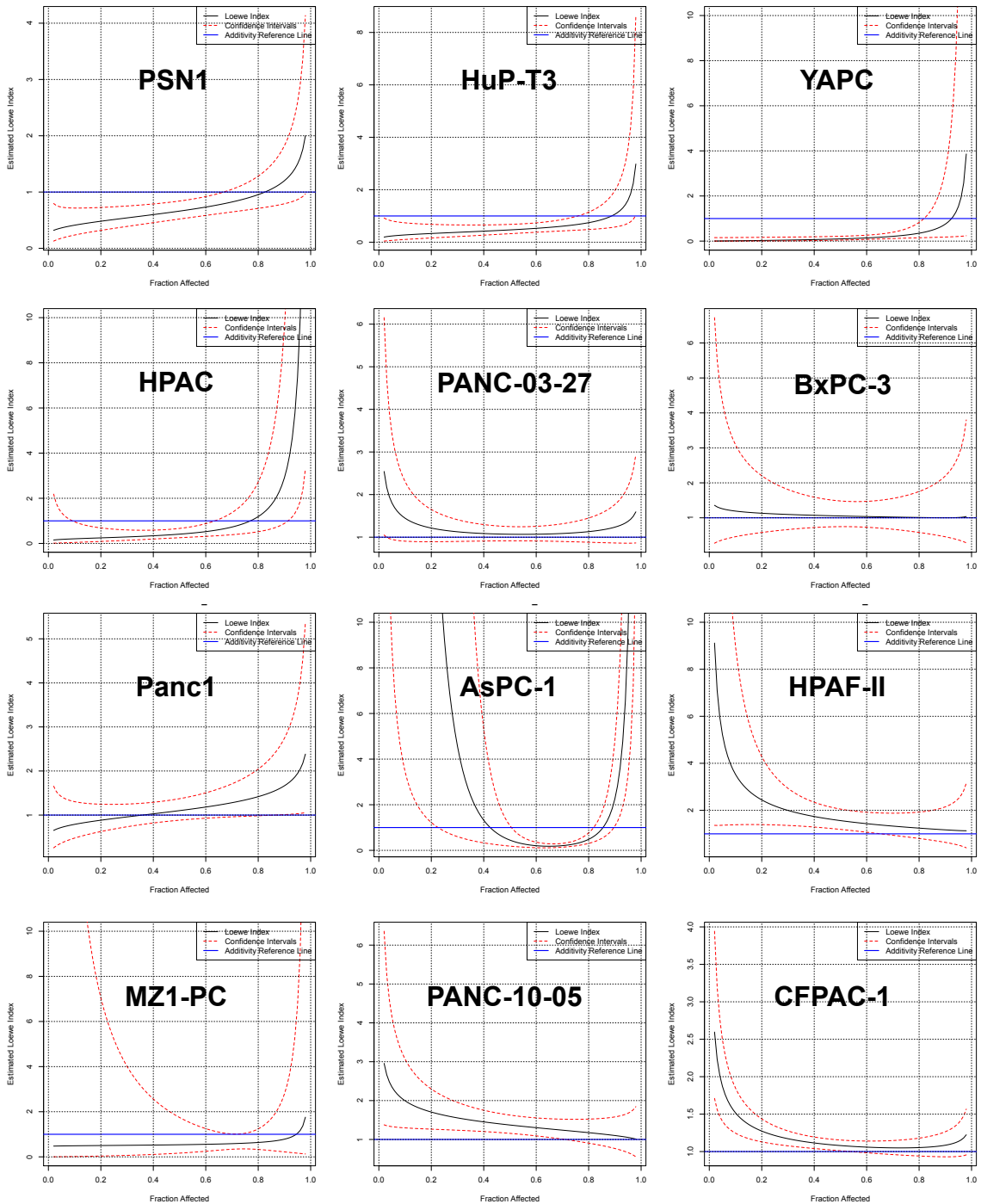


Figure S4. BMS-754807/PD-0332991 combination indices in the PDAC cell lines.

The PDAC cell lines were treated with BMS-754807, PD-0332991 or their fixed-ratio (1:10) combination over an 8-point, 128-fold concentration range and cell viability was determined after 72 h. The mixlow R package was used to fit dose-response curves for the single agent and combination treatment and to estimate Loewe synergy indices. Synergy plots show Loewe indices per fraction of cells affected by the combination treatment: <1 indicates synergism, $=1$ indicates additivity (blue line) and >1 indicates antagonism with a 95% confidence (red dashed lines). Results are from at least two independent replicates.

Supplementary Figure S5

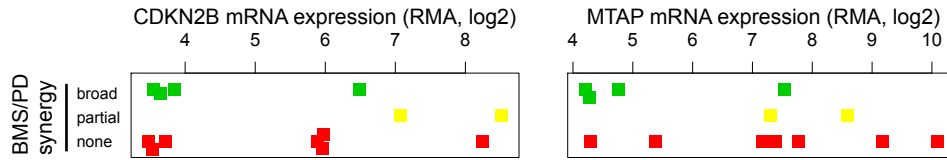


Figure S5. *CDKN2B* and *MTAP* mRNA expression in PDAC cell lines.

mRNA expression values (RMA, log₂) are from the Broad-Novartis Cancer Cell Line Encyclopedia database and shown for the PDAC cell lines with broad (green), partial (yellow) or no (red) synergy of BMS-754807 and PD-0332991.

Supplementary Figure S6

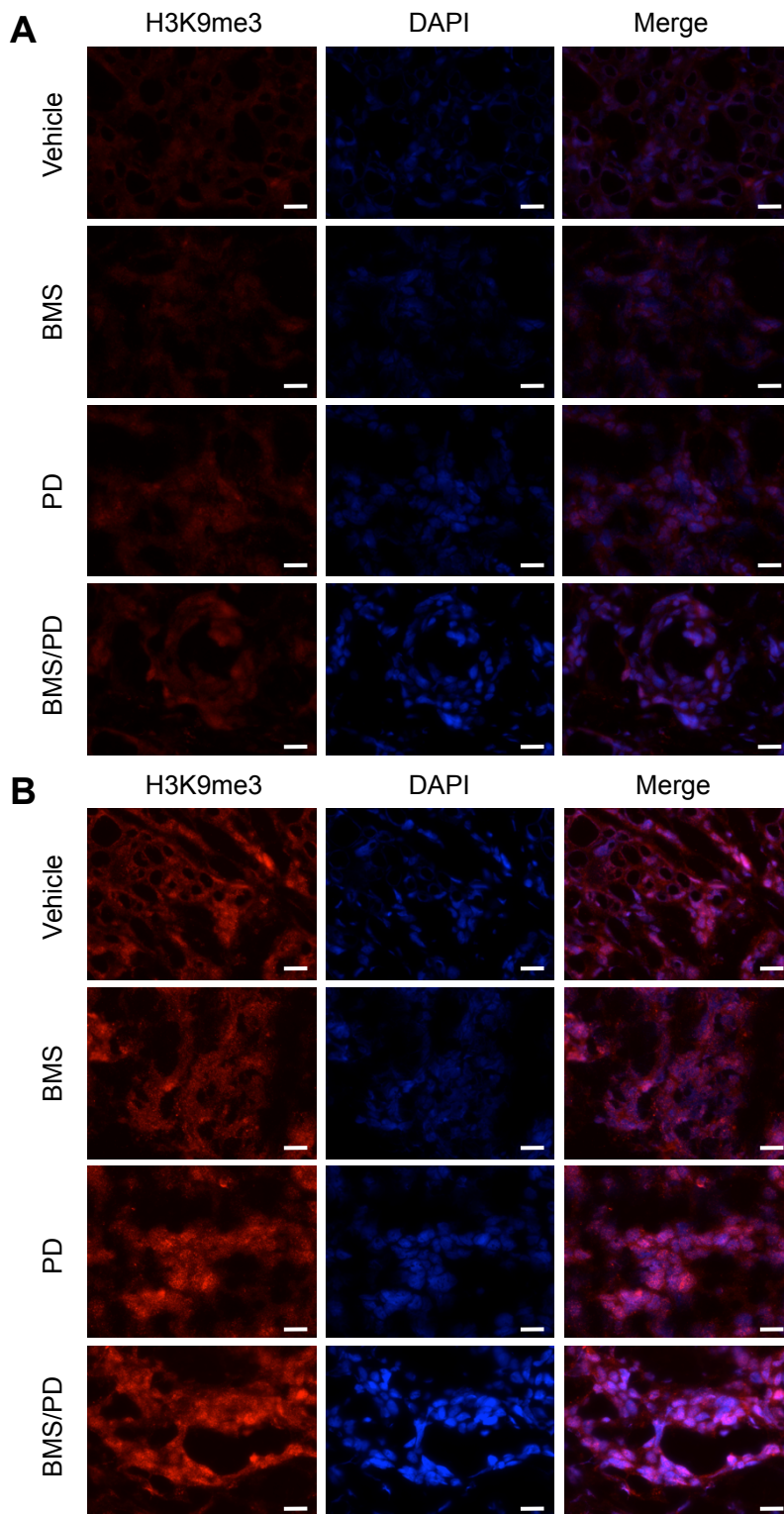


Figure S6. H3K9me3 staining of YAPC xenograft tumors.

Cryosections of the xenograft tumors that had been treated with BMS-754807 (BMS), PD-0332991 (PD), their combination (BMS/PD) or vehicle as described in the Materials and Methods were stained for H3K9me3 (red) and DNA (DAPI, blue) after completion of the treatment study. Images are representative. Scale bar 20 μ m. (A) Short and (B) long exposures.

Supplementary Figure S7

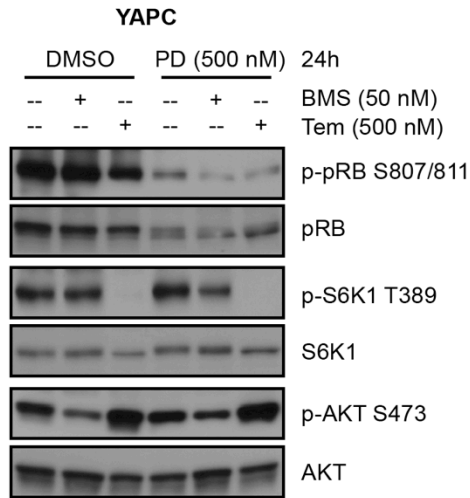


Figure S7. Drug combinations enhance inhibition of pRB phosphorylation by CDK4/6 inhibitor.

YAPC cells were treated for 24 h with 500 nM PD-0332991 (PD) or DMSO control in combination with 50 nM BMS-754807 (BMS), 500 nM temsirolimus (Tem) or DMSO and cell lysates were probed with the indicated antibodies. Comparable results were observed in four independent experiments; a representative Western blot is shown.

Supplementary Figure S8

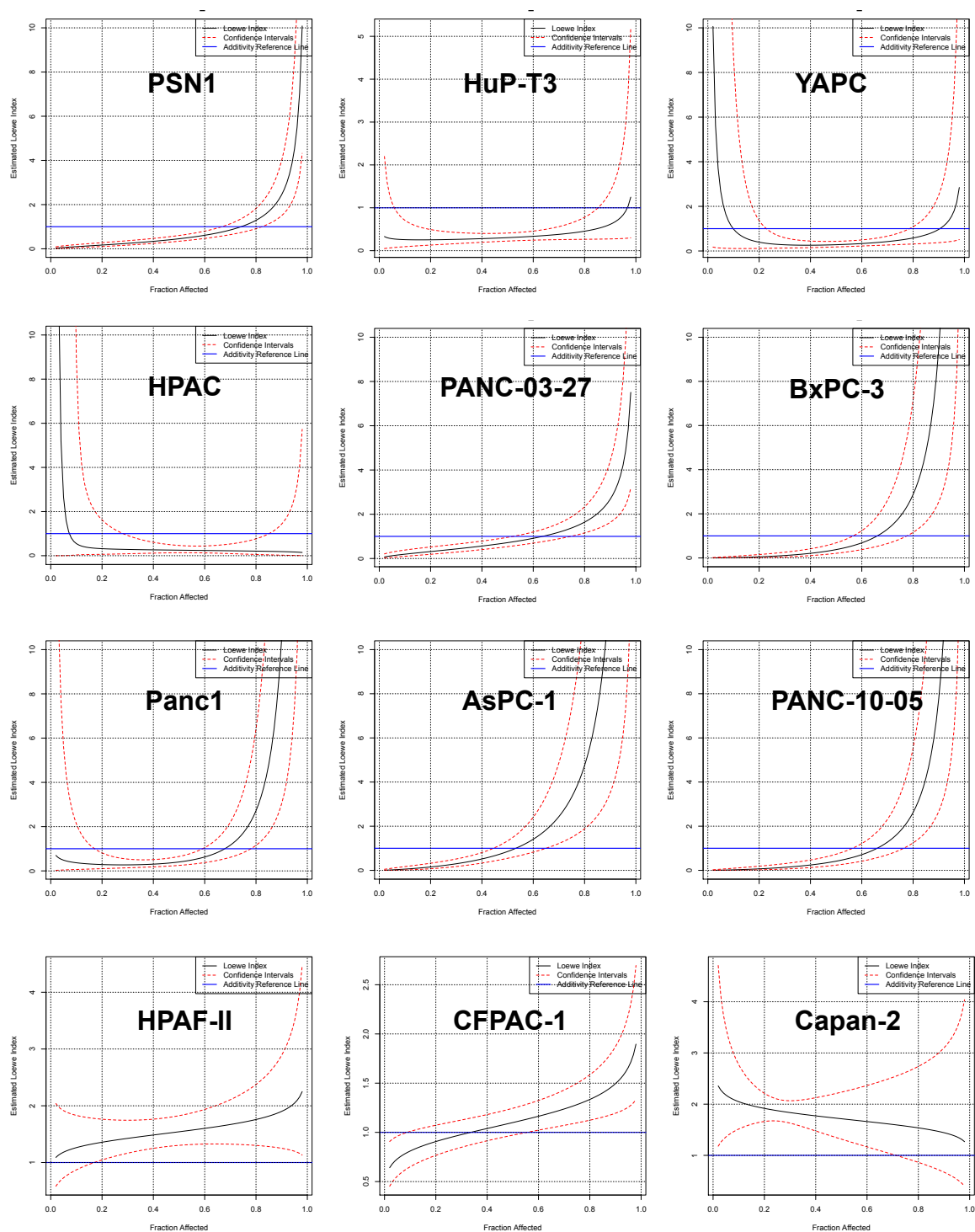


Figure S8. Temsirolimus/PD-0332991 combination indices in the PDAC cell lines.

The PDAC cell lines were treated with temsirolimus, PD-0332991 or their fixed-ratio (1:1) combination over an 8-point, 128-fold concentration range and cell viability was determined after 72 h. Synergy plots show Loewe indices per fraction of cells affected by the combination treatment: <1 indicates synergism, =1 indicates additivity (blue line) and >1 indicates antagonism with a 95% confidence (red dashed lines) (N=2).